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Radiolytic X-Ray Footprinting of the TATA Binding Protein

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ABSTRACT: Millisecond exposure of protein containing solutions to a synchrotron X-ray beam, produces hydroxyl radicals by radiolysis that preferentially oxidize Cys, Met, Phe, Tyr, Trp, Pro, His and Leu amino acid side chains. The protein footprinting technique is being applied using LC/MS to quantitate both the unoxidized proteolytic peptides of the protein and their radiolytic products.

The TATA binding protein (TBP) is required by each of the three RNA polymerases for the initiation of gene transcription. In performing its biological function, TBP binds to DNA and to the other proteins required to recruit a specific RNA polymerase. “Protein footprinting” is being used to explore the solution structure of the native Saccharomyces cerevisiae TBP. S. cerevisiae TBP contains a total of 15 Phe residues, four of which are located within the DNA-binding surface. Although this protein self-associates, initial experiments have been conducted under conditions where the protein is monomeric. The solvent accessibilities determined by radiolytic footprinting differ from those calculated from a monomer extracted from the co-crystal structure of the C-terminal domain TBP (which crystallized as a dimer). Preliminary results indicate that only a subset of the accessible and potentially reactive residues in the N-terminal subdomain of the C-terminal core domain display rates of hydroxyl radical modification commensurate with a high level of solvent accessibility. In addition, few residues of N-terminal domain are highly reactive despite the general belief that this ‘non-essential’ domain is unstructured in the absence of interactions with other proteins. An analysis of the accessibility of TBP residues as a function of DNA binding is also being conducted.